REMARKS

A. Status of the Claims

Claims 63-74 are currently pending. Claims 64 and 68-70 are withdrawn, claims 63, 65-67, and 71 stand rejected, and claims 72-74 are objected to as depending from non-allowed claims. Claim 63 and 66 are currently amended and claim 65 is canceled.

The amendment to claim 63 includes adding the limitations that were previously in claim 65, and clarifying that the fusions between the interactor domains and the enzyme fragments are at the newly created 5' and 3' ends at the breakpoint. The amendment to claim 66, merely updates the dependency from 65 (now canceled) to claim 63. No new matter is added with this amendment.

1. Clarification of claim language

The Examiner contends that "nothing in the Applicants' claims requires a particular type of fusion (i.e. 5' or 3'). Specifically, the Examiner states that "Applicants' claims merely require that an N-terminal fragment and a C-terminal fragment be used regardless of how they are connected to the interactor domains." (see, Office Action page 13, penultimate paragraph).

The Applicant disagrees with the Examiner's interpretation of the claims, but in an effort to expedite prosecution of the application the Applicants have amended claim 63 to more expressly set forth that one interactor domain is fused to the C-terminus of the N-terminal fragment and the other interactor domain is fused to the N-terminus of the C-terminal fragment. This language makes clear that the present application claims a single configuration in which the interactor domains can be fused to the β-lactamase fragments.

B. Rejection of Claims 63, 65 and 67 under 35 U.S.C. §103(a)

Claims 63, 65, and 67 stand rejected as *prima facie* obvious in view of Michnick *et al.*, ("Michnick"), Blau *et al.*, ("Blau") and Pieper *et al.*, ("Peiper").

Michnick is relied on as teaching, *inter alia*, a genus of fragment complementation fusion polypeptides. Michnick does not teach the use of a Class A β-lactamase enzyme, or a fragment complementation system where the first and second class A β-lactamase protein break-

points are within 10 amino acids in either direction from a junction between 2 amino acid residues of a loop between elements of secondary structure. **Blau** is cited as teaching, *inter alia*, the use of a Class A β-lactamase in a fragment complementation system like the one used by Michnick. **Pieper** is relied on as providing explicit examples showing that a class A β-lactamase may be "functionally reconstituted."

The Examiner asserts that the combined references of **Blau and Pieper** teach, the exact point at which a class A β-lactamase can be cleaved to form N-terminal and C-terminal fragments and yet still retain biological activity.

The Examiner contends that a *prima facie* case is established because 1) Michnick, in light of Blau and Pieper teach all of the elements of the claims, and 2) a skilled artisan reading Michnick would be motivated to use a class A β-lactamase because of the criteria found in the paragraph bridging columns 9 and 10 in Michnick, and 3) a skilled artisan would have a reasonable expectation of success because Blau explicitly states that a β-lactamase can be used in complementation systems and Pieper provides explicit examples showing that a class A β-lactamase may be functionally reconstituted.

As discussed in more detail below, the Examiner has not established a proper *prima facie* case of obviousness because 1) Michnick, alone or combined with the other cited references, does not teach or suggest all of the elements of the invention as presently claimed; and 2) the Examiner has not indicated where in the cited references a skilled artisan would find motivation to combine the references and arrive at the Applicants' invention. Specifically, to design a class A β-lactamase enzyme fragment complementation system in which a 1st and 2nd breakpoint are located within 10 amino acids in either direction from a junction of 2 amino acids located in a solvent exposed loop between elements of secondary structure *and* wherein <u>both</u> interactor domains are fused to the newly created ends at the breakpoints, as presently claimed.

1. Burden of Proof in Establishing Prima Facie Obviousness

The Examiner bears the burden of establishing a *prima facie* case of obviousness. *In re Rijckaert*, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993); *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). Only if this burden is met does the burden of

coming forward with rebuttal arguments or evidence shift to the applicant. *Rijckaert*, 9 F.3d at 1532, 28 USPQ2d at 1956. When the references cited by the Examiner fail to establish a *prima facie* case of obviousness, the rejection is improper and will be overturned. *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988)." See *In re Deuel*, 51 F.3d 1552, 34 USPQ2d 1210, 1214 (Fed. Cir. 1995).

In order to establish a *prima facie* case of obviousness, the rejection must demonstrate that (1) the cited references teach all the claimed elements; (2) there is a suggestion or motivation in the prior art to modify or combine the reference teachings; and (3) a reasonable expectation of success. MPEP § 2143; *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991).

As explained in more detail below, the Examiner has not established a proper *prima facie* case of obviousness because 1) the cited references do not teach or suggest all of the elements as presently claimed, and 2) the Examiner has not indicated where the cited references provide a motivation to locate the breakpoints in a solvent exposed loop between elements of secondary structure and fuse *both* of the interactor domains to the newly created ends at the breakpoint in an $N^{bkp} + C^{bkp}$ configuration as presently claimed. (*See,* Footnote)[1]

2. The Art of Record Fails to Teach Each Claim Element

The Examiner cites Michnick as teaching "a genus of fragment complementation fusion polypeptides that encompass the Applicants' claimed invention (or at least overlaps in scope to a large extent)." (*See*, Office Action mailed March 9, 2006, page 3).

Specifically, the Examiner cites Michnick as disclosing:

> [A] protein "fragment complementation" system comprising a "first fusion product" and a "second fusion product" (i.e., a system) that contains two enzyme fragments… and also two molecular domains that are roughly equivalent in scope to the claimed interactor domains (e.g., see Michnick et al., claim 1; see also figure 1; see also Examples). Michnick also disclose the "reconstitution"

[1] There are only four possible positions at which the interactor domains can be fused to the enzyme fragments. These positions are: N^{nat}, which refers to a construct where the interactor domain is fused to the native NH_2 terminus of the N-terminal fragment (i.e. at the 5' end of the N-terminal fragment). N^{bkp}, which refers to a construct where the interactor domain is fused to the newly created COOH terminus at the breakpoint of the N-terminal fragment (i.e. at the 3' end of the N-terminal fragment). C^{nat}, which refers to a construct where the interactor domain is fused to the native COOH terminus of the C-terminal fragment (i.e. at the 3' end of the C-terminal fragment). C^{bkp}, which refers to a construct where the interactor domain is fused to the newly created NH2 terminus at the breakpoint of the C-terminal fragment (i.e. at the 5' end of the C-terminal fragment).

> of said first and second interactor domains to yield a functional enzyme...

However, Michnick combined with the other references does not teach or suggest a fragment complementation system in which the first and second breakpoints are located within 10 amino acids in either direction from a junction of 2 amino acids located in a solvent exposed loop *and* fusing *both* of the interactor domains to the newly created ends at the breakpoint in an N^{bkp} + C^{bkp} configuration as presently claimed.

The basic difference between the presently claimed invention and the general method as disclosed by Michnick in view of Blau and Pieper, is illustrated in **EXHIBIT 1**. The top half of the exhibit illustrates the Applicants invention as presently claimed using the proteins Fos and Jun as interactor domains. The fully functional β-lactamase protein is cleaved to form an N-terminal and a C-terminal fragment by introducing a first and second breakpoint located within 10 amino acids in either direction of 2 joining amino acids in a solvent exposed loop between elements of secondary structure. The interactor domains, Fos and Jun, are fused to the β-lactamase fragments in an N^{bkp} + C^{bkp} configuration at the breakpoints. Interaction between the interactor domains drives the reconstitution of the fragments to generate a functional β-lactamase enzyme.

The bottom half of Exhibit 1 shows the general method for a protein complementation assay (PCA) as taught by Michnick in view of Pieper and Blau, using the β-lactamase enzyme (*see*, Pieper and Blau) with the proteins Fos and Jun as interactor domains. The enzyme is rationally dissected to generate an N-terminal fragment and a C-terminal fragment. *See*, Pieper and criteria in Michnick (Col 3, line 66 to Col 4 line 9). The interactor domains are then fused to the 5'-ends of each fragment in an N^{nat} + C^{bkp} configuration. *See,* Michnick Col 4, lines 27-42. As shown in the illustration, it is hard to imagine how a functional β-lactamase protein could be functionally reconstituted by the interaction of the interactor domains when fused in an N^{nat} + C^{bkp} configuration as taught by Michnick in light of Blau and Pieper.

As discussed in more detail below, Michnick combined with the teachings of Blau and Pieper, does not teach or suggest all of the elements of the invention as presently claimed.

THE 2 MISSING CLAIM ELEMENTS ARE: A) LOCATION OF THE BREAKPOINTS AND B) CONFIGURATION OF THE INTERACTOR DOMAINS.

a) The cited references do not teach locating a first and second breakpoint within 10 amino acids in either direction from a junction of 2 amino acids within a solvent exposed loop.

The Examiner contends that Blau and Pieper "teach the exact point at which a Class A β-lactamase can be cleaved to form N-terminal and C-terminal fragments and yet still retain biological activity." *See*, page 5 of the Office Action. In particular, the Examiner refers to two constructs disclosed in Pieper stating that "the cp254 that was cleaved in a loop remote from the domain interface retained similar activity to the wild-type β-lactamase; see also cp228 construct which shows rates that are 0.5-1% of the native enzyme against some substrates and 10-fold faster than the wild-type against a third generation cephalosporin." *See*, bottom page 5 to top page 6 of the Office Action. The Examiner thus concludes that "Pieper provide explicit examples showing that a class A β-lactamase may be functionally reconstituted" *See,* page 7 of the Office Action.

The Applicants disagree. Pieper does not disclose a protein fragment complementation system. Rather, Pieper discloses a uni-molecular circularly permutated molecule in which the native N and C-termini were fused and a new N and C-termini were created. Pieper merely shows that this molecule with a new N and C-termini has some degree of functional activity. This is irrelevant to the present application because two separate fragments that were subsequently rejoined at the breakpoint were never created.

Therefore, Michnick in view of Pieper and/or Blau, does not teach or suggest the element of locating a first and second breakpoint "within 10 amino acids in either direction from a junction between 2 amino acid residues...within a solvent exposed loop between elements of secondary structure" as presently recited in claim 63.

b) The cited references do not teach or suggest fusing the interactor domains in an $N^{bkp} + C^{bkp}$ configuration.

Michnick discloses **a general strategy** for protein complementation systems in which the interactor domains are placed in an $N^{nat} + C^{bkp}$ configuration. Specifically, Michnick states that "the chosen fragments are subcloned, and ***to the 5' ends of each***, proteins that either are

known or thought to interact are fused." *See*, Michnick *et al.*, Col. 4, lines 27-42 (emphasis added). This is very different from the fragment complementation system as presently claimed, which teaches fusion of both the interactor domains to the newly created ends at the breakpoint in an N^{bkp} + C^{bkp} configuration.

Notably, the Examiner alleges that Michnick is not limited to the N^{nat} + C^{bkp} configuration specifically citing Michnick "[i]t is crucial to understand that these assays will only work if the fused, interacting proteins catalyze the reassembly of the enzyme." *See*, page 12, of the Office Action, and Col 4, lines 38-40 of Michnick. The Examiner interprets this passage as implying that Michnick "does not require a particular fusion but, rather, any fusion that will allow the enzyme fragments to reassemble into an active form." *See*, page 12 of the Office Action.

The Applicants contend that the passage cited by the Examiner is taken out of context. Specifically, the cited passage occurs near the end of a paragraph describing the general teachings of the PCA system, in which Michnick clearly and unambiguously states that:

> The present application explains the rationale and criteria for using a particular enzyme in a PCA. ***Fig. 1 shows a general description of a PCA.*** The gene for a protein or enzyme is rationally dissected into two or more fragments. Using molecular biology techniques ***the chosen fragments are subcloned, and to the 5' ends of each*** protein [i.e. N^{nat} + C^{bkp} configuration], proteins that either are known or thought to interact are fused...Reassembly of the probe, protein or enzyme from its fragments is catalyzed by the binding of the test proteins to each other...***It is crucial to understand that these assays will only work if the fused, interacting proteins catalyze the reassembly of the enzyme.*** That is, observation of reconstituted enzyme activity must be a measure of the interaction of the fused proteins. (Emphasis added Col 4 lines 27-42).

When the passage cited by the Examiner is read in context of the entire paragraph, it is clear that Michnick was not referring to the notion of any fusion of the interacting domains that would allow for the functional reconstitution of the enzyme (as contended by the Examiner). Rather, Michnick was emphasizing that the functional reconstitution of the enzyme is dependent on the interaction of the fused proteins. This reinforces the importance of the specific requirements of the general teaching as disclosed by Michnick in the passage quoted *supra*, that the interacting

proteins be fused to the 5'-ends of the enzyme fragments *i.e.*, in an $N^{nat} + C^{bkp}$ configuration unless indicated otherwise.

Blau, also teaches the $N^{nat} + C^{bkp}$ configuration and therefore does not cure the defect in Michnick. Rather, Blau describes the construction of β-galactose fragments in which the interactor domains (FKBP12 and FRAP) are **fused in an $N^{nat} + C^{bkp}$ configuration.** *See*, page 35, lines 25-27; and Fig 2B and Fig 7B of Blau.

Furthermore, the Examiner appears to confuse the criteria for selecting a breakpoint with that of selecting the points of fusion for the interactor domains. For example, the Examiner contends that Michnick provides:

> extensive guidance for selecting enzyme breakpoints that will insure the proper reassembly of the enzyme...1) The fragments should result in subdomains of continuous polypeptide; that is the resulting fragments will not disrupt the subdomain structure of the protein, 2) the catalytic and cofactor binding sites should all be contained in one fragment, and 3) resulting new N-and C-termini should be on the same face of the protein to avoid the need for long peptide linkers... *See*, bottom page 12 to top of page 13 of the Office Action.

The Examiner then concludes that "...the three factors cited above make clear that *a particular type of fusion is not required* and a person of ordinary skill in the art would not interpret it as such" (emphasis added). *See*, bottom page 12 to top page 13 of the Office Action.

The Applicants disagree. The factors as cited above, while possibly providing some guidance in selecting the position for the breakpoint, *do not* provide any guidance as to the attachment of the interactor domains to the resulting fragments.

A skilled artisan reading Michnick in the broadest interpretation, in light of the other cited references, might recognize that the general teaching of Michnick is applicable to a variety of proteins (possibly even β-lactamase) and that interactor domains other than leucine zippers may be used. However, a skilled artisan would not recognize from the combined teachings of the cited references the distinct advantages that result from locating the breakpoint within a flexible or solvent exposed loop between elements of secondary structure and fusing the interactor domains to the newly created ends at the breakpoint in an $N^{bkp} + C^{bkp}$ configuration as presently claimed.

The Examiner has failed to establish a proper *prima facie* case of obviousness because Michnick alone or in combination with the cited references does not teach or suggest all of the elements of the invention as presently claimed. In light of the above, the Applicants respectfully request that the Examiner withdraw the rejection.

3. There is No Motivation in the Prior Art to Modify the Referenced Fusion Constructs

Even if the cited references disclosed all the elements of the claimed invention, which the Applicants contest, there is still no motivation provided in the cited prior art to modify the fusion constructs of Michnick to arrive at Applicants' invention. The cited references do not disclose any deficiency in the reported constructs that would motivate one skilled in the art to fuse the interactor domains in an $N^{bkp} + C^{bkp}$ configuration as presently claimed. Thus, the Examiner's *prima facie* case rests on the belief that any random combinations of fusions of the interactor domains to the enzyme fragments are equal. They are not.

Here, the Applicants recognized and applied creativity in selecting the sterically superior $N^{bkp} + C^{bkp}$ configuration that is not taught or suggested by the prior art. The application of random combinations for fusing the interactor domains, as suggested by the Examiner, is not a substitute for motivation. In fact, the only motivation to modify the referenced fusion constructs is provided in Applicants' disclosure. To establish a proper *prima facie* case of obviousness, the teaching or suggestion to make a modification to the prior art must be found in the prior art, not in Applicants' disclosure.

Importantly, the Examiner has not contended that one of skill in the art would be motivated to alter the placement of the interactor domains to a $N^{bkp} + C^{bkp}$ configuration as presently claimed, but rather that one of skill in the art would have been motivated to use a Class A β-lactamase enzyme because Michnick states that:

> In designing a protein-fragment complementation assay (PCA), we sought [i.e. were motivated] to identify an enzyme for which the following is true: 1) An enzyme that is relatively small and monomeric, 2) for which structural and functional information exists, 3) for which simple assays exist for both *in vivo and in vitro* measurement, and 4) for which overexpression in eukaryotic and prokaryotic cells has been demonstrated."

(e.g., see Office Action page 14 and Michnick paragraph bridging col 9-10).

While the Examiner cites these factors as pointing toward the use of class A β-lactamase, there is nothing in the above passage that would motivate one of skill in the art to fuse the interactor domains in an N^{bkp} + C^{bkp} configuration as presently claimed.

Because none of the cited references teach or suggest the N^{bkp} + C^{bkp} configuration, it appears that the Examiner is improperly using hindsight reconstruction to argue that a skilled artisan would have recognized the steric advantages of the N^{bkp} + C^{bkp} configuration as presently claimed.

The Examiner, however, has not pointed to anywhere in the prior art that would motivate a skilled artisan to localize a first and second breakpoint within 10 amino acids in either direction from a junction between 2 amino acids located within a solvent exposed loop between elements of secondary structure of a β-lactamase enzyme *and* fuse *both* of the interactor domains to the newly created ends at the breakpoint in an N^{bkp} + C^{bkp} configuration as presently claimed. In view of the above, the Examiner has not established a proper *prima facie* case of obviousness and the Applicants respectfully request that the rejection be withdrawn.

a) Pieper Teaches Away from fusing the interactor domains to the newly created ends in an N^{bkp} + C^{bkp} configuration as presently claimed, because Pieper fused the native ends.

In addition to the arguments as presented above, Pieper teaches away from the invention as presently claimed. As discussed *supra*, Pieper teaches a uni-molecular circularly permutated protein in which the native N and C-termini are fused and a new N and C-termini are introduced in a loop of the enzyme between domains of secondary structure. A skilled artisan would not recognize Pieper as teaching a suitable breakpoint for fusion of interactor domains to the newly created termini as presently claimed because Pieper fuses the native N and C-termini. Thus, when Pieper is combined with Michnick, a skilled artisan would see a fragment complementation construct in which the native N and C-termini are fused *and* the interactor domains are fused to the newly created ends at the breakpoint. Because Pieper fuses the native N and C-termini, the additional fusion of interactor domains to the newly created ends at the breakpoint would place an additional constriction on the enzyme when the interactor domains interact. It is doubtful that such

an enzyme construct with multiple constrictions (fusion of the native ends *and* interaction of the interactor domains fused to the newly created ends at the breakpoint) would even be functional. Therefore, Pieper teaches away from the invention as presently claimed.

A skilled artisan, reading Michnick in light of Blau and Pieper would not appreciate the steric advantages of localizing the breakpoint to a solvent exposed loop and fusing *both* of the interactor domains to the newly created termini at the breakpoints. It is the steric advantages provided by this precise combination of locating the breakpoints in a solvent exposed loop and fusing both of the interactor domains to these newly created termini in an N^{bkp} + C^{bkp} configuration that makes the present invention patentable over the cited references. (See, Exhibit 1).

The Examiner has not established a proper *prima facie* case for obviousness because 1) none of the prior art references alone or in combination teach or suggest all of the elements of claim 63 as presently recited, and 2) the Examiner has not indicated where the prior art provides a motivation to modify the references to arrive at the presently claimed invention.

Claim 65 is canceled and claim 67 depends indirectly from claim 63 and therefore includes all of the limitations of the independent claim 63. In light of the above, the Applicants respectfully request that the obviousness rejection be withdrawn.

C. Rejection of Claims 63, 65, 66 and 71 under 35 U.S.C. §103(a).

Claims 63, 65, 66, and 71 stand rejected as *prima facie* obvious in view of Michnick *et al.*, Blau *et al.*, Pieper *et al.*, Moore *et al.* ("Moore"), and Maveyraud *et al.* ("Maveyraud").

The arguments as presented above with regard to Michnick, Blau and Pieper are applicable to this rejection as well. Furthermore, Moore and Maveyraud fail to cure the defects of Michnick, Blau and Pieper because Moore and Maveyraud are merely cited for the proposition that "TEM-1 β-lactamase is a good reporter." *See*, Official Action dated June 27, 2005 at page 12.

Claims 66, and 71 depend from independent claim 63, and therefore include all of the limitations of claim 63. In light of the above, the Applicants respectfully request that the Examiner withdraw the rejection.

D. Double Patenting Rejection

The Examiner has rejected claims 63, 65-67 and 71 under the judicially created obviousness-type double patenting as allegedly unpatentable over claims 63-65 of U.S. Patent Application No. 09/526,106 in view of Michnick and Blau. Claims 63, 65-67 and 71 stand rejected under the judicially created obviousness-type double patenting as allegedly unpatentable over claims 1, 12, and 13 of U.S. Patent Application No. 10/330,811.

Applicants note that United States Patent App. No. 09/526,106 has been expressly abandoned thereby mooting the provisional double patenting rejection with respect to that application. Furthermore, claims 1, 12, and 13 of United States patent Application No. 10/330,811 are currently non-elected as a result of the Restriction Requirement dated December 19, 2005, and the Election of Group III (Claims 21-57) dated February 21, 2006. Therefore, the provisional double patenting rejection to U.S. Pat. App. No. 10/330,811 is moot also.

In light of the above, the Applicants respectfully request that the Examiner withdraw the rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are nonobvious over the cited references. The withdrawal of all obviousness rejections is respectfully requested.

Respectfully submitted,

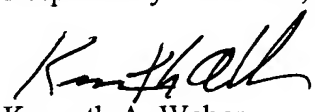

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EXHIBIT 1